DIFFERING EFFECTS OF IN VIVO HYPEROXIA ON ERYTHROCYTES*

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The use of oxygen under high pressure (OHP) for medical and surgical purposes[1-6] and 100% oxygen for space cabin atmospheres[7] have stimulated renewed interest in, and provided a unique opportunity for study of, the biochemical and functional changes that occur during exposure of animals or humans to high oxygen environments. The possibility occurred to us that a hyperoxic environment might enhance oxidation of compounds in excess of that which would occur under normal physiologic conditions. A specific consideration was the possible *in vivo* peroxidation of unsaturated fatty acids.

Unsaturated fatty acids readily autoxidize *in vitro* to form lipid peroxides[8-11]. The reaction takes place nonenzymatically in the presence of oxygen and ferrous ions. Studies in this laboratory and in others have linked *in vitro* peroxidation of erythrocyte lipid and hemolysis[12-19]. Some observations had suggested that oxidation of unsaturated fatty acids might occur *in vivo*[12,20,21]. However, the occurrence of lipid peroxidation *in vivo* had not been unequivocally demonstrated, and therefore, its biologic significance was not established.

Previous studies carried out in this laboratory[22-23] suggested that hemolysis occurring in mice exposed to OHP resulted from peroxidation of erythrocyte lipid. Evidence suggesting that a similar hemolytic mechanism could occur in humans was obtained from our findings in a patient who developed hemolytic anemia after a brief period of exposure to OHP[24]. The studies reported here demonstrate (1) *in vivo* peroxidation of RBC

lipid, (2) its occurrence prior to RBC damage and lysis, and (3) other changes occurring as a result of O_2 per se rather than an effect of lipid peroxidation.

IN VIVO PEROXIDATION OF LIPID

Methods

Mice. Male and female strain DBA/2 mice (6 to 9 months old, average weight 25 g) were used in all experiments. For each experiment 20 mice of comparable age, sex, and weight were exposed to hyperoxia. Ten of these were taken from a group of mice that had been maintained on a tocopherol-deficient test diet for a minimal period of 6 weeks. The other ten, which had been fed a standard chow preparation, were each injected intraperitoneally with 0.5 mg of alpha-tocopherol acetate 0.5, 3 or 18 hours before OHP exposure. In each experiment an equal number of control mice of comparable age, sex, and dietary status but without exposure to hyperoxia were studied. The weight of mice in each study group did not differ appreciably.

Exposure to hyperoxia. Mice were placed in metal cages that had been coated with a saline-glycerine solution (fire safety precaution) and which contained no food, water, or combustible material. The test cages were placed in a hyperbaric chamber. This chamber had a volume of 12 cubic feet and provided constant circulation of the gaseous environment with continual flushing by 100% oxgyen and absorption of expired CO_2. Chamber pressure was brought to 60 pounds per square inch absolute pressure with 100% oxygen over a period of 5 to 10 minutes and was maintained for 1.5 hours. No CO_2 could be demonstrated at several intervals tested with a micro-Scholander gas analyzer. Slow stepwise decompression was carried out over 20 minutes.

Within 1 hour after removal from the chamber each mouse was exsanguinated by cutting exposed axillary blood vessels. This method (one of several tested) enabled us to obtain the greatest volume of blood (average, 1 ml) per mouse. Blood was collected in heparinized pipettes and immediately cooled to 4°C.

Routine and special hematologic studies. Microhematocrits, reticulocyte counts, and Heinz body preparations were performed on individual and pooled blood samples[25]. Plasma was examined for evidence of gross hemoglobinemia.

Lipid peroxide determinations. Lipid peroxides in erythrocytes were determined by measuring the pink chromogen (absorbance maximum 535 mμ) formed by the reaction of 2-thiobarbituric acid (TBA) with a breakdown product of lipid peroxides, malonylaldehyde[30,26]. Erythrocytes from mice in each study group were washed twice in physiologic saline. Then 0.16 or 0.2 ml portions of washed erythrocytes were mixed well with 1.5 ml 10% trichloracetic acid. The mixture was filtered through Whatman No. 1 paper. Thiobarbituric acid (0.67% in water) was added to portions of the filtrate (usually 0.6 or 0.8 ml) in a ratio of 1.2 to 1. The mixture was heated in a boiling water bath for 15 minutes, then cooled to room temperature. Absorption spectra were taken and the absorbance at 525 mμ recorded.

Lipid peroxide levels in plasma were determined by combining 1 ml of plasma with 1 ml of 10% trichloroacetic acid and filtering the mixture through Whatman No. 1 paper. One ml of the filtrate was mixed with 1.2 ml of the thiobarbituric acid solution and lipid peroxides were determined as outlined in the preceding paragraph.

Since it has not been possible to prepare a standard solution of unsaturated fatty acid peroxides, a standard absorption curve for malonylaldehyde was prepared using 1,1,3,3-tetraethoxypropane (TEP), a compound that hydrolyzes to 1 mole of malonylaldehyde and 4 moles of ethanol[27]. With this curve as a standard an absorbance of 0.1 in the TBA reaction was calculated to be equivalent to 8 nmoles of malonylaldehyde. Although many other aldehydes and ketones give some color with the TBA reagent, they fade rapidly and have different absorption maximums or low extinction coefficients[28].

Saturated fatty acids do not peroxidize. Malonylaldehyde is derived primarily from those unsaturated fatty acids which contain three or four unsaturated bonds, such as arachidonate and linolenate[29]. Since these represent only a fraction of the total unsaturated fatty acids in naturally occurring lipids, this method measures only a portion of the total peroxidized unsaturated fatty acids. For example, Hochstein and Ernster found that malonylaldehyde levels accounted for only approximately 5% of the total oxygen consumed during peroxidation of lipids in rat liver microsomes[30]. Most investigators agree, however, that this method may be used as a measure of lipid peroxidation[31-34].

Results

The effect of *in vivo* OHP on mouse red cells is shown in Table I. Before OHP exposure no significant differences of hematologic indexes were noted between tocopherol-deficient and tocopherol-supplemented mice. Mice that had been maintained on the tocopherol-deficient diet for 4 months had normal hematocrits and showed no evidence of hemolysis before OHP exposure. During OHP, hemolysis (fall of hematocrit and marked hemoglobinemia) occurred

in tocopherol-deficient mice. Whereas hematocrit values varied among individual mice, each tocopherol-deficient mouse exposed to OHP showed clear-cut evidence of hemolytic anemia. No evidence of hemolysis during OHP was noted in mice supplemented with tocopherol 0.5, 3 or 8 hours before exposure to OHP. Neither sex nor age (in the range studied, 6 to 10 months) affected lytic sensitivity to OHP. When blood of individual mice was studied, no correlation was noted between central nervous system manifestations and severity of the hemolysis in the tocopherol-deficient group.

Red cells in Wright's-stained blood films showed moderate size and shape variations with some fragmentation of cells only in tocopherol-deficient mice exposed to OHP. No significant numbers of spherocytes were seen in any of the blood films.

Lipid peroxides were present in erythrocytes obtained from tocopherol-deficient mice immediately after exposure to OHP. None were found in erythrocytes from tocopherol-deficient mice not exposed to OHP. No lipid peroxides were detected in erythrocytes of tocopherol-supplemented mice either before or after OHP. Plasma of tocopherol-deficient mice exposed to OHP contained only trace amounts of lipid peroxides.

Further studies were carried out to determine whether the lipid peroxides found in erythrocytes of tocopherol-deficient mice exposed to OHP had been formed *in vivo* during exposure of the mice to OHP, or *in vitro* as the erythrocytes were manipulated in the presence of atmospheric oxygen. Erythrocytes of tocopherol-deficient mice formed large quantities of lipid peroxides *in vitro* and were lysed when exposed to 1) bubbled oxygen at 37° for 6 to 12 hours, 2) 100% oxygen at 60 pounds per square inch absolute

pressure at 37°C for 1 and 12 hours, 3) 0.1% hydrogen peroxide at 37°C for 3 hours, or 4) ultraviolet radiation (42 cm below two Westinghouse Sterilamps G1 T8 in round bottom quartz flasks for 6 hours at 25°C.

To determine the effect of prior in vivo tocopherol - deficient mice were each given 0.5 mg of alpha-tocopherol acetate intraperitoneally 1 hour before bleeding. Blood was collected in pipettes that had been rinsed with physiologic saline containing alpha-tocopherol emulsified in Tween-80 and physiologic saline (0.5 mg per ml), and all subsequent steps of the TBA test were performed with solutions containing alpha-tocopherol (0.5 mg per ml). When these red cells were subjected to the oxidant stresses listed above, each of which is capable of peroxidizing lipid, no significant lysis or lipid peroxidation occurred.

Since tocopherol as we used it had prevented in vitro lipid peroxidation by these agents, we reasoned that it should also prevent any in vitro peroxidation of lipid by atmospheric oxygen in erythrocytes of mice exposed to OHP. Accordingly, tocopherol-deficient mice were exposed to OHP in the routine manner and given 0.5 mg ip of alpha-tocopherol immediately after decompression. One hour later blood was collected in tocopherol-rinsed pipettes, and lipid peroxides were determined by using solutions containing alpha-tocopherol and saline mixtures as described above. As shown in Table II, there were no differences in lipid peroxide levels between the tocopherol-deficient mice that were bled immediately after OHP and those which were given the tocopherol after OHP but before bleeding. No additional lipid peroxide formation occurred when erythrocytes of these animals were subsequently exposed to H_2O_2, oxygen, and ultraviolet

radiation. Thus, the lipid peroxides found in erythrocyte of tocopherol-deficient mice exposed to OHP must have been formed in vivo.

SEQUENCE OF EVENTS AFTER PEROXIDATION OF RBC LIPID

Methods

Mice. Mice similar to the previous study were used.

Exposure to hyperoxia. Exposure to hyperbaric oxygen was carried out in a cylindrical hyperbaric chamber 6 inches in internal diameter, 16 inches long, with a total volume of 450 cubic inches. In each experiment ten tocopherol-deficient mice were placed directly into the chamber, which was then flushed at normal atmospheric pressure with 100% oxygen for 5 minutes. Compression with 100% oxygen to 60 pounds per square inch, absolute, (4 atmospheres), was carried out over a period of 15 minutes, and pressure was maintained for 60 minutes. Continual circulation of the gaseous environment was maintained throughout exposure by a constant influx of pure 100% oxygen, and by constant efflux at a rate of 10 liters per minute. Fifteen minutes were allowed for slow, stepwise decompression. Total exposure time to 100% oxygen was 95 minutes.

Studies of red cells. Blood was obtained from ether-anesthetized mice, immediately after exposure and at regular timed intervals thereafter, by severing axillary vessels which had been surgically exposed. Hematocrits, reticulocyte counts, Wright's-stained blood films and Heinz body preparations were performed on blood collected in microhematocrit tubes from individual mice. Red cell counts were performed using a Model F-Coulter Counter.

Osmotic fragility of red cells was tested using the Osmotic Fragiligraph* as described by Danon[35]. Determinations were carried out within five minutes after the blood was obtained. Cumulative and derivative curves were inscribed in each study and values expressed as the salt concentrations at which 50% hemolysis occurred.

RBC lipid peroxides were determined on heparinized blood pooled from two mice as noted previously. Lipid peroxide determinations were performed on specimens of plasma and urine by the same method, using the same volumes.

Results

The course of hemolysis after exposure of the tocopherol-deficient mice to OHP is illustrated in Fig. 1. Immediately after exposure there was no evidence of gross hemolysis and the hematocrit was slightly higher ($P < .05$) than it had been prior to OHP. Ten minutes after exposure, while the mice were maintained at normal atmospheric conditions, hemolysis began, indicated by the appearance of visible hemoglobin in plasma and by a slight fall in hematocrit ($P < .025$). Thirty minutes after exposure, the hematocrit had fallen to 30%, plasma was bright red, and hemoglobinuria was observed. Hemolysis was progressive, with the hematocrit falling to 12% 4 hours after OHP, and all animals died 5-6 hours after OHP. During the hemolytic phase reticulocytes increased to a high of 18%.

The concomitant changes of erythrocyte morphology and osmotic fragility are summarized in Table III. Immediately after OHP red cell morphology appeared normal, the MCV was not significantly altered but there

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an increase of osmotic fragility. Thereafter a significant increase of MCV and an increasing number of spherocytes in blood films was accompanied by an additional increase in cumulative osmotic fragility, apparently due to a distinct population of osmotically fragile red cells as identified by the derivative curve inscribed by the Fragiligraph. During the phase of most rapid hemolysis (from 20 minutes to 2 hours post-OHP) large numbers of ghosted and/or fragmented red cells were observed in blood films. Disappearance of significant numbers of spherocytes (i.e. at 2 hours post-OHP) was accompanied by a decrease of MCV and a return of cumulative osmotic fragility to normal with a single population of cells as determined by the derivative curve. No methemoglobin and no Heinz bodies were present in RBCs of these mice before OHP, or at any time after exposure to OHP.

The relationship between lipid peroxides and lysis is shown in Fig. 2. Before exposure neither RBCs nor plasma contained measureable levels of lipid peroxides. Immediately after exposure and before hemolysis began relatively large quantities of lipid peroxides were found in red cells but not in plasma. At 10 minutes after OHP, when hemolysis apparently began there was a significant decrease in red cell lipid peroxide levels which occurred concomitantly with the initial appearance of lipid peroxides in plasma. Thereafter there was a progressive decrease of both red cell and plasma lipid peroxide levels. Four hours after exposure to OHP, neither RBCs nor plasma contained detectable amounts of lipid peroxides.

One hour after exposureurine contained a substance which on acid hydrolysis and heating with 2-thiobarbituric acid formed a pink pigment which absorbed maximally at 535 mu and was, presumably, malonylaldehyde.

EFFECT OF HYPEROXIA ON RED CELLS OF NORMAL MICE

Methods

Mice. Male and female strain DBA/2 mice (all between 4 and 6 months of age) were maintained on standard chow diets. Mice of the same age, sex, and average weight were used in each study. For special studies some mice received 0.5 mg alpha-tocopherol acetate each, given daily for 5 days prior to study.

Exposure to hyperoxia. Groups of 10 mice were exposed to 100% oxygen at pressures of 60 psia (lb./sq. in. absolute) for 90 minutes. The chamber used had a volume of 424 cubic inches. The chamber was first flushed for 5 minutes at 15 psia and the pressure was gradually increased to the desired level over a 15 minute period. Stepwise decompression was completed in 15 minutes. For studies involving larger numbers of mice, a larger chamber was used. This chamber had a volume of 12.56 cubic feet.

Studies of blood. All mice were exsanguinated by severing the right axillary vessels. For most studies blood was collected immediately using heparinized pipettes and the sample tubes were kept at 4°C. Subsequent studies were carried out on individual or pooled blood samples. Hematocrits, hemoglobins, reticulocyte counts and osmotic fragilities were determined by standard methods.

Erythrocyte glycolytic intermediates. These were determined by modifications of the methods described by Shafer and Bartlett[36] and Bartlett[39]. Fifty ml of samples of pooled heparinized blood from 100 mice were used for each study. Erythrocytes were washed three times with cold physiologic saline. Packed erythrocytes (12.5 ml) were added with constant vigorous mixing to 2 volumes of 10% trichloroacetic acid (TCA). After centrifugation and filtration of the supernate the precipitate was re-extracted with

2 volumes of 5% TCA. After combining extracts, TCA was removed with four extractions of 2 volumes of ether. The ether was then removed by bubbling nitrogen through the solutions. After neutralization extracts were passed through a 1 x 20 column of Dowex-1 x 8-formate resin. The columns were then eluted with a linearly increasing concentration of 0 to 3.5 N ammonium formate buffer, pH 3.0 at a rate of 3-4 ml/min. Fractions were collected in 10 ml volumes and each fraction was analyzed for total phosphorus content. In addition, compounds were identified by elution position as determined by standard applications previously performed in this laboratory and by specific analytic methods[38,39]. Quantities of compounds determined in this system were expressed as micromoles phosphorus/ml of erythrocytes.

Erythrocyte adenosine triphosphate (ATP). ATP determinations were made by a modification of the method of Beutler and Baluda utilizing the firefly-luminescence method[40]. All chemicals and reagents were prepared exactly as described by them. Blood taken from individual animals (0.8 ml) was mixed immediately with 0.2 ml cold ACD and determinations were carried out within 10 minutes. One-tenth of blood (diluted 4:1 V/v with ACD) was added to 2.9 ml of iced tris-borate buffer in a graduated 15 ml test tube. The hemolysate was immediately placed in a boiling water bath for 5 minutes and then transferred to an ice bath after restoring the volume to 3 ml with distilled water. Two-tenths ml of hemolysate were added to 2 ml of ice-cold diluted firefly extract and the tube was inverted 5 times. After exactly 1 minute, the emitted fluorescence was read at 560 mu. The ATP content was determined by reference to a standard curve and was expressed as umoles/Gm of hemoglobin.

Adenosine diphosphate (ADP) and adenosine monophosphate (AMP). These determinations were carried out according to the methods of Lipman[41] and Bucher[42], using DPN-DPNH linked changes in forward and backward reactions between phosphoenolpyruvate and lactate.

Splenectomies were carried out under ether anesthesia through a left upper quadrant abdominal incision. The animals received tetracycline-HCl (.02 ml/Gm) daily, postoperatively for 5 days. All studies of these animals were carried out 2 weeks after splenectomy.

Results

In control animals exposed to OHP there were increases in hematocrit and osmotic fragility. (Table IV) These effects were not eliminated by splenectomy or prior tocopherol supplementation. In fact, splenectomized mice had an increase of MCV and a greater per cent increase of hematocrit. The effect of OHP on RBC ATP and ADP is shown in Table V. ATP content was consistently and significantly increased while ADP content was concomitantly decreased.

The effect of 100 per cent oxygen at 60 psia for 1 hour on phosphorylated erythrocyte glycolytic intermediates of chow-fed mice is shown in Table VI. No evidence of hemolysis was noted. As determined in this laboratory by the chromatographic method cited, levels of phosphate compounds in mouse erythrocytes were the same in two different groups of normal mice. The most significant variations from normal in the mice exposed to OHP were increases in erythrocyte ATP and hexose monophosphate compounds, with concomitant decreases in levels of fructose-1,6-diphosphate, the triose-phosphates, and triphosphopyridine nucleotide (TPN). The remainder of the measured compounds were not significantly altered.

These observations suggested a block between fructose-6-phosphate and fructose-1,6-diphosphate, a step mediated by the enzyme phosphofructokinase. The effect of OHP on RBC phosphofructokinase activity is shown in Table VII. Fifty per cent decreases of enzyme activity were observed after OHP.

DISCUSSION

Although the clinical and histopathologic features of oxygen toxicity have been described in detail, the primary mechanism of cell damage by high oxygen tensions has not been elucidated. Aside from studies relating to erythropoiesis, the *in vivo* effect of increased oxygen tension on erythrocytes has received little attention. Its relevance to human clinical situations had not been considered until recently when several volunteers maintained in simulated space capsule environments (100% O_2 at low atmospheric pressures) developed evidences of red cell damage (increased osmotic fragility) and hemolysis (fall of hemoglobin and evaluation of reticulocytes and indirect-reacting bilirubin), and one patient developed hemolytic anemia after a brief exposure to OHP. The latter patient's red cells were similar to those of tocopherol-deficient mice with regard to increased lytic sensitivity to H_2O_2, increased lipid peroxide formation by H_2O_2, and their *in vivo* sensitivity to hyperoxia. Whether his susceptibility reflected a tocopherol-deficient state or some alteration of fatty acid content in his erythrocytes was not decided. Many other studies were helpful only in ruling out various possibilities.

Work in other laboratories and our own studies had linked high oxygen tensions, erythrocyte lysis and lipid peroxidation *in vitro*, and previous studies in this laboratory had suggested their relationship *in vivo*. The

present studies established the fact that peroxidation of erythrocyte lipid can occur *in vivo* during exposure to oxygen under high pressure. These effects were noted only in mice fed a tocopherol-deficient diet.

A frequent criticism of earlier studies of lipid peroxidation had been the failure to consider the ability of atmospheric oxygen to peroxidize unsaturated fatty acids *in vitro*. Since determinations of lipid peroxide content always involved manipulations during which tissue was exposed to atmospheric oxygen, it always seemed possible that any lipid peroxides found could have been formed *in vitro*. Our observation that levels of erythrocyte lipid peroxides were not decreased when alpha-tocopherol was administered after OHP, but before exsanguination (a maneuver we proved effective in preventing *in vitro* lipid peroxidation) established their formation *in vivo*. They were not formed as a result of hemolysis, since lipid peroxides were demonstrated in remaining intact erythrocytes.

Subsequent results of this investigation have helped to clarify the relationship between peroxidation of RBC lipid and hemolysis in tocopherol-deficient mice exposed to OHP. Proof that peroxidation of RBC lipid preceded hemolysis came from the finding that RBCs contained large quantities of lipid peroxides immediately after exposure to OHP, before hemolysis began. That the onset of overt hemolysis (first discernible hemoglobinemia) and the attendant initial decrease in RBC lipid peroxide content coincided with the first appearance of lipid peroxides in plasma, suggested that lipid peroxides were released into plasma from damaged RBCs.

The sequence of events in changing red cell morphology and osmotic fragility suggested that a particular population of cells had been damaged, undergone sphering and were ultimately destroyed. The marked hemoglobinemia and hemoglobinuria and the large numbers of ghosts and red cell fragments indicated that hemolysis was predominantly intravascular.

An unexpected finding in these studies was the speed and magnitude of the reticulocyte response after hemolysis. Morphologically these were classic reticulocytes, most of which appeared to be very primitive. Preliminary morphologic studies of the bone marrow have revealed an obvious increase in the number of erythropoietic elements, maximal at the time of peak reticulocytosis, 30 minutes after exposure to OHP. This finding, also unexpected, is not currently understood and will require additional study.

The finding that hemolysis can be delayed in onset, beginning after exposure to OHP, broadens current concepts of oxygen toxicity. We had previously observed a similar pattern of hemolysis in a human after exposure to OHP, and had postulated that this was a form of delayed oxygen toxicity. Other investigators have also alluded to the possibility that some manifestations of oxygen toxicity might be delayed in onset, beginning <u>after</u> exposure to OHP. However, the current investigation has provided the first direct demonstration of a form of delayed oxygen toxicity.

In contrast to these results, no hemolysis occurred in chow-fed mice (those resistant to peroxidation of lipid). There were changes of RBC membrane (increased MCV and osmotic fragility) and phosphorylated glycolytic intermediates. These changes reflected changes in cells themselves rather than a shift of cell populations. The metabolic effects were shown in part at least to result from inhibition of the RBC enzyme phosphofructokinase.

SUMMARY

1. Hemolytic anemia induced by exposure to OHP is associated with peroxidation of RBC lipid.

2. RBC lipid peroxidation occurs *in vivo* before the onset of hemolysis and is responsible for hemolysis.

3. Hemolysis mediated by peroxidation of RBC lipid can begin after exposure to OHP and progress under normal atmospheric conditions.

4. During OHP, O_2 *per se* increases RBC ATP, hematocrit, and osmotic fragility.

5. The increase of ATP is an effect of cell metabolism, probably via inhibition of phosphofructokinase.

6. Therefore, although O_2 *per se* produced transient metabolic and physical changes in red cells, irreversible damage and lysis only occurred when peroxidation of lipid has taken place.

LEGENDS FOR FIGURES

Figure 1. Hematologic effect of hyperbaric oxygen on tocopherol-deficient mice. Each point represents the mean of 4 to 10 determinations (each in duplicate) carried out on individual mice. The bars above and below each point indicate ± one standard deviation.

Note that hemolysis began after exposure to hyperoxia, while the mice remained at normal atmospheric conditions.

Figure 2. Relationship between lipid peroxide levels and lysis. The hematocrit values are those of Figure 2. For lipid peroxide levels the points represent mean values of from 2 to 8 separate determinations (each in duplicate) using pooled blood of two mice and the bars indicate ± one standard deviation.

TABLE I

Hematologic Values and Lipid Peroxide Levels in Erythrocytes from Mice*

Study Group†	Hematocrit	Per cent Reticulocytes	Appearance of Plasma	Lipid Peroxides‡
Tocopherol-deficient (14)	45-50	0.2-1.5	Normal	0
Chow-fed, tocopherol-supplemented(14)	44-51	0.2-1.6	Normal	0
Tocopherol-deficient + OHP (14)	14-24	9-16	Bright red	36-50
Chow-fed, tocopherol-supplemented + OHP (14)	47-50	0.2-1.4	Normal	0
Tocopherol-deficient, tocopherol-supplemented +OHP (5)	46-49	0.1-1.2	Normal	0

*Values were obtained from pooled blood samples of ten mice in each experimental group.

† The number after each group indicates the number of experiments; +OHP designates those mice exposed to oxygen under high pressure.

‡ Millimicromoles malonylaldehyde per milliliter erythrocytes.

TABLE II

Lipid Peroxide Levels in Mouse Erythrocytes*

Mouse Study Group	Lipid Peroxides
Tocopherol-deficient	0
Tocopherol-deficient + OHP	32-39
Tocopherol-deficient + OHP (given tocopherol before bleeding)	34-38
Tocopherol-deficient + OHP (given tocopherol before bleeding, blood collected and washed in tocopherol-saline mixture)	32-41
Tocopherol-supplemented (before and after OHP)	0

*Values were obtained on pooled blood of ten mice in each experimental group, and the range of three separate experiments is given.

Millimicromoles malonylaldehyde per milliliter erythrocytes.

TABLE III

Changes in Erythrocytes After Exposure to OHP

Time	RBC Morphology	MCV	Osmotic Fragility
Before OHP	Normal	45 ± 3	.43 ± 0 
After OHP			
Immediately	Unchanged	46 ± 1	.47 ± 0 
10 min.	Moderate size and shape variation 20-30% spherocytes	55 ± 2	.495 ± .005 
20 min.	Same as 10 min.		.50 ± 0 
30 min.	60-70% spherocytes Moderate no. of ghosts and fragments, moderate polychromasia	52 ± 3	.50 ± .008
1 hour	Same as 30 minutes		.50 ± .005 
2 hours	Many ghosts and fragments Few spherocytes Moderate polychromasia	43 ± 4	.42 ± .005 
4 hours	Almost no ghosts Moderate size and shape variation Marked polychromasia	44 ± 2	.38 ± 0 

TABLE IV

Effect of In Vivo Hyperoxia on Chow-Fed Mice

Study Group	Hct. %	Retics. %	Osmotic Fragility	MCV u^3
Control	44 ± 2	3.0	.46 ±.01	46 ± 4
Control + OHP	50 ± 3	3.3	.49 ± .01	47 ± 4
Splenectomy	41 ± 3	3.2	.45 ± .01	46 ± 4
Splenectomy + OHP	49 ± 4	3.4	.49 ± .03	51 ± 4
Tocopherol Supplemented + OHP	50 ± 2	3.1	.49 ± .02	---

TABLE V

Effect of OHP on Red Cell ATP and ADP

Study Group	ATP uM/gm Hgb.	ADP uM/gm Hgb.
Control	5.3 ± .3	.37 ± .05
Control +OHP	7.3 ± .4	.19 ± .06

Values represent means ± 1 SD

TABLE VI

Changes of Phosphate Compounds of Erythrocytes in Chow-Fed Mice Exposed to 100 Per Cent Oxygen at 60 Psia for 1 Hour

Compound	Normal Mice #1	Normal Mice #2	OHP Mice	PerCent*
Inorganic phosphorus(P)	.14	.11	.12	96
AMP	Trace	Trace	Trace	--
Adenosine diphosphate (ADP)	.34	.32	.32	99
Adenosine triphosphate (ATP)	.24	.21	.60	270
Diphosphopyridine nucleotide (DPN)	.24	.24	.22	92
Triphosphopyridine nucleotide (TPN)	.24	.26	.13	52
Fructose, 1-6, diphosphate	1.28	1.27	.72	56
Diphosphoglyceric acid	7.3	7.9	8.7	115
Triose phosphates	.21	.22	.10	47
Hexose mono-phosphates	.24	.26	.80	320

Quantities of metabolites are expressed as umoles phosphorus/ml of erythrocytes. Data of normal mice represent levels in pooled blood of 2 separate groups of mice. OHP mice denotes blood from thos mide exposed to hyperoxia.

* Concentration of mice exposed to OHP divided by concentration of normal control mice.

TABLE VII

Effect of Hyperoxia on RBC Phosphofructokinase

Study Group	Enzyme Activity Eu/ul cells/minute
Control	22.9 ± 2.0
Control + OHP	12.2 ± 1.2

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